

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

1. (Currently amended) A method for determining the concentration of a double stranded nucleic acid molecule in a biological sample, comprising:

(a) obtaining a biological sample from a subject;

(b) assaying a first portion of the sample for the concentration of any unhybridized single stranded component of the double stranded nucleic acid molecule under conditions suitable to determine the concentration of the unhybridized single stranded component in the sample;

(c) processing a second portion of the sample under conditions suitable for any double stranded nucleic acid molecule present in the sample to ~~dissociate~~ disassociate into one or more single stranded components;

(d) assaying the second portion for the concentration of any ~~dissociated~~ disassociated single stranded component of the double stranded nucleic acid molecule under conditions suitable to determine the concentration of the ~~dissociated~~ disassociated single stranded component in the sample; and

(e) comparing the concentration of the unhybridized single stranded component to the concentration of the ~~dissociated~~ disassociated single stranded component under conditions suitable to determine the concentration of the double stranded nucleic acid molecule in the sample.

2. (Original) The method of claim 1, wherein the processing in (c) comprises heating the second portion at about 85 to about 95 degrees C for about 5 to about 30 minutes.

3. (Original) The method of claim 1, wherein the assaying in (b) comprises:

(i) combining the first portion of the sample with a capture oligonucleotide affixed to a surface under conditions suitable for the capture oligonucleotide to specifically hybridize with a first portion of the unhybridized single stranded component;

(ii) washing the surface under conditions suitable to remove any unbound portion of the double stranded nucleic acid molecule;

(iii) adding a detection oligonucleotide to the surface of (ii) under conditions suitable for the detection oligonucleotide to specifically hybridize with a second portion of the unhybridized single stranded component;

(iv) washing the surface under conditions suitable to remove any unbound detection oligonucleotide;

(v) adding a reporter molecule to the surface of (iv);

(vi) washing the surface under conditions suitable to remove any unbound or unreacted reporter molecule;

(vii) measuring the amount of the bound or reacted reporter molecule; and

(viii) determining the concentration of the unhybridized single stranded component by comparing the amount of the reporter molecule with a standard curve.

4. (Original) The method of claim 1, wherein the assaying in (b) comprises:

(i) combining the first portion of the sample with a detection oligonucleotide under conditions suitable for the detection oligonucleotide to specifically hybridize with a first portion of the unhybridized single stranded component;

(ii) combining the product of (i) with a capture oligonucleotide affixed to a surface under conditions suitable for the capture oligonucleotide to specifically hybridize with a second portion of the unhybridized single stranded component;

(iii) washing the surface under conditions suitable to remove any unbound detection oligonucleotide complex;

(iv) adding a reporter molecule to the surface of (iii);

(v) washing the surface under conditions suitable to remove any unbound or unreacted reporter molecule;

(vi) measuring the amount of the bound or reacted reporter molecule; and

(vii) determining the concentration of the unhybridized single stranded by comparing the amount of the reporter molecule with a standard curve.

5. (Currently amended) The method of claim 1, wherein the assaying in (d) comprises:

(i) combining the second portion of the sample with a capture oligonucleotide affixed to a surface under conditions suitable for the capture oligonucleotide to specifically hybridize with a first portion of the ~~dissociated~~ disassociated single stranded component;

(ii) ~~(ii)~~ washing the surface under conditions suitable to remove any unbound portion of the double stranded nucleic acid molecule;

(iii) adding a detection oligonucleotide to the surface of (ii) under conditions suitable for the detection oligonucleotide to specifically hybridize with a second portion of the ~~the~~ ~~dissociated~~ disassociated single stranded component;

(iv) washing the surface under conditions suitable to remove any unbound detection oligonucleotide;

(v) adding a reporter molecule to the surface of (iv);

(vi) washing the surface under conditions suitable to remove any unbound or unreacted reporter molecule;

(vii) measuring the amount of the bound or reacted reporter molecule; and

(viii) determining the concentration of the ~~the dissociated~~ disassociated single stranded component by comparing the amount of the reporter molecule with a standard curve.

6. (Currently amended) The method of claim 1, wherein the assaying in (d) comprises:

(i) combining the second portion of the sample with a detection oligonucleotide under conditions suitable for the detection oligonucleotide to specifically hybridize with a first portion of the ~~dissociated~~ disassociated single stranded component;

(ii) combining the product of (i) with a capture oligonucleotide affixed to a surface under conditions suitable for the capture oligonucleotide to specifically hybridize with a second portion of the ~~dissociated~~ disassociated single stranded component;

(iii) washing the surface under conditions suitable to remove any unbound detection oligonucleotide complex;

(iv) adding a reporter molecule to the surface of (iii);

(v) washing the surface under conditions suitable to remove any unbound or unreacted reporter molecule;

~~(v)~~ (vi) measuring the amount of the bound or reacted reporter molecule; and

~~(vi)~~ (vii) determining the concentration of the ~~dissociated~~ disassociated single stranded component by comparing the amount of the reporter molecule with a standard curve.

7. (Currently amended) The method of claim 1, wherein (c) further comprises removing any single stranded component of the double stranded nucleic acid molecule from the sample that can ~~competitively~~ competitively bind to the other single stranded component of the double stranded nucleic acid molecule.

8. (Currently amended) The method of claim 7, wherein (c) comprises:

- (i) heating the sample to about 90 degrees C for about 10 minutes;
- (ii) treating the sample with a streptavidin conjugated complementary oligonucleotide sequence that binds to the single stranded ~~siNA~~ component; and
- (iii) removing the single stranded component from the assay.

9. (Original) The method of claim 1, wherein the double stranded nucleic acid molecule comprises a nucleic acid molecule that mediates RNA interference.

10. (Original) The method of claim 3, wherein the nucleic acid molecule that mediates RNA interference is a short interfering nucleic acid molecule (siNA).

11. (Currently amended) The method of claim 9, wherein ~~wherein~~ the double stranded nucleic acid molecule comprises a nucleic acid molecule that mediates RNA interference against VEGF RNA.

12. (Currently amended) The method of claim 9, wherein ~~wherein~~ the double stranded nucleic acid molecule comprises a nucleic acid molecule that mediates RNA interference against VEGFR1 RNA.

13. (Currently amended) The method of claim 9, wherein ~~wherein~~ the double stranded nucleic acid molecule comprises a nucleic acid molecule that mediates RNA interference against VEGFR2 RNA.

14. (Currently amended) The method of claim 9, wherein ~~wherein~~ the double stranded nucleic acid molecule comprises a nucleic acid molecule that mediates RNA interference against Hepatitis C Virus (HCV) RNA.

15. (Currently amended) The method of claim 9, wherein ~~wherein~~ the double stranded nucleic acid molecule comprises a nucleic acid molecule that mediates RNA interference against

Hepatitis C Virus (HBV) RNA.

16. (Currently amended) The method of claim 9, wherein ~~wherein~~ the double stranded nucleic acid molecule comprises a nucleic acid molecule that mediates RNA interference against HIV RNA.

17. (Original) The method of claim 10, wherein the siNA comprises one or more 2'-deoxy-2'-fluoro nucleotides.

18. (Original) The method of claim 10, wherein the siNA comprises one or more 2'-O-methyl nucleotides.

19. (Original) The method of claim 10, wherein the siNA comprises one or more inverted deoxyabasic moieties.

20. (Original) The method of claim 10, wherein the siNA comprises one or more 2'-deoxy nucleotides.